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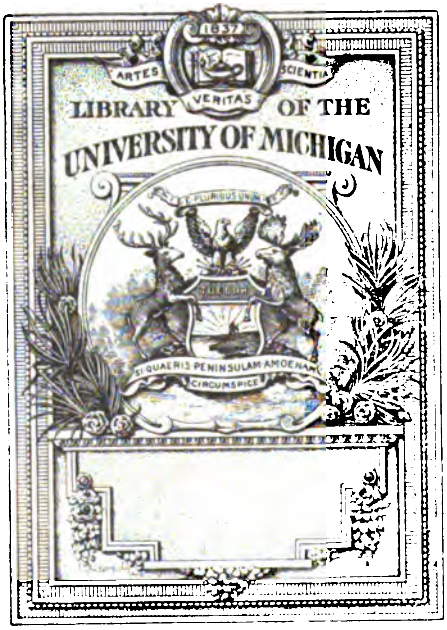
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THE EFFECT OF BONE ASH IN THE DIET ON THE GASTRO-INTESTINAL CONDITIONS OF DOGS

DISSERTATION
Submitted in partial fulfilment of the requirements for the degree of Doctor
of Philosophy, in the faculty of Pure Science
of Columbia University.

By

ADFRED PEIRCE LOTHROP, A. M.

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THE EFFECTS OF BONE ASH IN THE DIET ON THE GASTRO-INTESTINAL CONDITIONS OF DOGS.

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I. INTRODUCTION.

METABOLISM experiments on dogs, as usually conducted, are beset by many difficulties when the objects of the investigations require that the urine and feces be kept separate for analysis and that the feces be collected as quantitatively as possible. When an animal is fed a diet consisting of hashed meat, cracker meal, lard, and water in the proportions ordinarily given in this laboratory,[1] nearly all of the food is digested and assimilated so that the fecal discharges are comparatively infrequent (usually at intervals of three or four days), but the character of the discharges under such conditions is usually decidedly diarrheal. When such a condition exists, there is very apt to be a mingling of the urine and

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[1] Fifteen gm. of meat, 4 gm. of cracker meal, 3 gm. of lard, and 35 c.c. of water per kilo of body weight.

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feces if the animal is confined in a cage and the urine collected automatically in a receiver at the bottom. Such a mingling makes accurate analysis of the excreta impossible and may ruin the experiment.

Many observations have been made in this laboratory on the thorough assimilation of the food in dogs on the diet already referred to, and also on the fluidity, offensive odor, and troublesome nature of the fecal discharges from such dogs.[2] From every other standpoint, however, such a diet is satisfactory.

As the number of workers in metabolism experiments increased in this laboratory, the difficulties incident upon collecting and preparing the feces for analysis became more and more prominent, and some method of relief was sought. When dogs receive food containing bone, the feces eliminated by them are solid, gray in color, and practically free from odor. They dry quickly in the air and can be easily ground to a powder closely resembling calcium phosphate. The organic constituents of the bone are, of course, digested, and the insoluble bone salts, mostly calcium compounds, which remain unabsorbed, are eliminated almost wholly in the feces. Numerous investigators had previously employed indigestible products mixed with the diet to give solidity to the feces, but it did not seem desirable in carefully conducted experiments to continually administer material which is never contained in the natural diet of the animal and which may have undesirable mechanical effects during its passage through the digestive tract. This criticism cannot, however, apply to the use of bone, for it is a material which dogs naturally ingest, and which their digestive processes apparently dispose of without any deleterious effects. The administration of bone as such, however, presents many difficulties. It would be impossible to feed it in daily masses of uniform composition unless pulverized. In that condition it would be apt to contain much organic matter whose removal by digestion might not be uniform, but whose retention might cause irregular contributions to the feces and thus disturb the daily metabolic events.

The only possibility seemed to be the thoroughly roasted bone ash containing only the inorganic constituents of the bone with a slight increase in carbonate. Accordingly, a series of trials was made with bone ash, and its great advantages were clearly demonstrated. The ash seemed to have absolutely no deleterious effects; the animals made no objections to ingesting even large quantities mixed with their food; and the fecal matter was practically inodorous, white, and dry, and could be quantitatively collected for analysis. About five years ago the first mention of its systematic use was casually made in a report of an investigation,[3] and shortly afterward a more detailed account of the general advantages of its use in the diet of dogs, especially in metabolism experiments, was presented.[4]

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[2] MEAD and GIES: This journal, 1901, v, p. 104; also GIES and collaborators: Biochemical researches, 1903, i (Reprint No. 21).

Since that time bone ash has been added regularly to the food of practically all the animals used in experiments in this laboratory and has given nothing but satisfaction. It has even been given advantageously in milk to kittens to prevent diarrhea. Ordinarily 1 gm. per kilo of body weight has been added to the food mixture, but five times as much has often been given without causing any unfavorable effects. Besides preventing diarrhea, moderate amounts of bone ash in the diet considerably increase the bulk of the excrementitious matter and make its discharge more frequent and regular, defecation usually occurring daily. Thus intestinal putrefaction is minimized by regular evacuation of the intestinal tract. Occasionally dogs eat their own feces, especially if particularly odoriferous. Such an event in a metabolism experiment causes very disturbing conditions. The bone ash feces are so brittle and inodorous that they incite no inclination in the dog to ingest the material. Only two out of several hundred dogs have shown any tendency thus to dispose of "bone-ash feces."

Steel and Gies[5] have investigated the influence of bone ash on the elimination of calcium and phosphate in the urine. Their results seem to show that in spite of the large amounts of calcium and phosphate ingested, in the form of the bone ash calcium phosphate, there is no change in the urinary excretion of calcium and no increase in the elimination of phosphorus (presumably also of phosphate). As much as 50 gm. of the bone ash were given with one day's food to a dog weighing only 6 kilos, and, although this is about eight times the amount usually given, the animal handled it readily and very little, if any, was absorbed.

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[3] TALTAVALL and GIES: This journal, 1903, ix, p. xvi; also GIES and collaborators: Biochemical researches, 1903, i, p. 59.

[4] GIES: Proceedings of the American Physiological Society, This journal, 1904, x, p. xxii.

[5] STEEL and GIES: This journal, 1907, xx, p. 350.

It was also found that even freshly voided, strongly acid urine will not appreciably dissolve any of the bone ash from feces, fragments of which are sometimes flushed into the receiver by the urine if the fecal matter is not immediately removed from the cage. Bone ash (2 gm.) in contact with strongly acid urine (150 c.c.) for four hours yielded to the urine only 0.0003 gm. of CaO, an amount which is within the unavoidable experimental error of method.[6]

In the course of an investigation on the influence of magnesium sulphate on metabolism, Steel[7] found that bone ash will not appreciably offset the cathartic effect of magnesium sulphate, except in a purely mechanical way, by adding bulk to the excrementitious matter. Recovery from the cathartic effects of large doses of magnesium sulphate was almost immediate when the administration of the magnesium sulphate was discontinued and that of the bone ash continued.

In spite of the large amounts of phosphate administered in the bone ash added to the diet, Hawk and Gies[8] in hemorrhage experiments on dogs, and Berg and Welker[9] in radium researches on dogs, have found it possible to maintain true phosphorus balances as satisfactorily as those of nitrogen and sulphur. All that was required was special alertness and care in the collection of the excreta.

In view of the systematic use of bone ash in all the metabolism experiments in this laboratory, and the probability that its employment will become general if it is devoid of deleterious influences, it is desirable to inquire more fully into the effect of bone ash on gastro-intestinal conditions, and to investigate the chemical changes, if any, that it undergoes in passing through the digestive tract. What are the effects of bone ash on intestinal putrefaction? Is digestion modified unfavorably? Is absorption hindered? Are any of the bone ash constituents eliminated in the urine? Is the secretion of intestinal mucus increased? Is general metabolism affected? To answer these and other questions this investigation was undertaken.

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[6] STEEL and GIES: *Loc. cit.*, p. 356.

[7] STEEL: Journal of biological chemistry, 1908, v, p. 98.

[8] HAWK and GIES: This journal, 1904, xi, p. 171.

[9] BERG and WELKER: Journal of biological chemistry, 1906, ii, p. 371.

II. METABOLISM EXPERIMENTS.

1. **General description of the metabolism experiments.** *Animals and environment.* — The metabolism experiments were performed on 4 healthy dogs, weighing from 7 to 12½ kilos. They were confined, while under observation, in cages devised in this laboratory and recently described.[10] The metabolism experiments were conducted along the lines usually employed in this laboratory.[11]

Food. — The daily food consisted of a mixture of hashed lean beef, cracker meal, lard, and water. The raw beef was taken from large supplies preserved in a frozen condition.[12] The cracker meal was obtained from large quantities of the commercial product kept in large glass bottles with ground glass stoppers. The lard used was Armour's best pure leaf lard, purchased in three-pound pails and kept in a refrigerator. Ordinary tap water was used. In the first two experiments the dogs received their daily rations in one portion at 12.45 P. M. and in the third and fourth experiments at 2.30 P. M.

Periods, weights. — In general each experiment was divided into four periods: a preparatory period during which the animal became accustomed to its surroundings and received its regular diet, but during which no analyses were made; a fore period and after period under normal conditions, and a middle dosage period during which bone ash was mixed with the food. These periods were of different lengths. In the first two experiments each day ended at 12.45 P. M. and in the second two at 2.30 P. M. The dogs were weighed just before being fed.

Collection of excreta. — The urine was collected daily just before feeding, and the feces were removed whenever noticed in the cage. As a rule, the cages were sprayed and well washed daily, and were cleaned especially thoroughly at the end of each period. The development of bacteria in the cage was thus kept as low as possible. The daily washings were used to dilute the urines to the uniform volume of 1 litre. The urine was preserved with powdered thymol. When necessary, the feces were desiccated over a water bath.

Analyses. — The total nitrogen of the meat and cracker meal was determined. In the *period* urines total ash, nitrogen, phosphorus, sulphur, and calcium were determined, and in the *daily* portions the ethereal sulphates. The period feces, after thorough grinding, sifting, and mixing, were analyzed for total nitrogen, ash, and fat (ether extraction). In two experiments the quantities of fecal lecithin were also determined.

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[10] GIES: This journal, 1905, xv, p. 403.

[11] MEAD and GIES: This journal, 1901, v, p. 106; GIES and collaborators: Biochemical researches, 1903, i, p. 419 (Reprint No. 21).

[12] GIES: This journal, 1901, v. p. 235; also GIES: Proceedings of the Society for Experimental Biology and Medicine, 1908, v, p. 27.

Analytical methods. — The following methods of analysis were employed: *Nitrogen:* Kjeldahl process, oxidation being effected by concentrated sulphuric acid aided by a little copper sulphate. *Phosphorus:* Liebig alkali fusion method and final weighing as magnesium pyrophosphate. *Sulphur:* Liebig alkali fusion method; also the fuming nitric acid method of Gill and Grindley.[13] *Ethereal sulphates:* Folin method.[14] *Fat:* Complete ether extraction in the Soxhlet apparatus. *Lecithin:* Calculated from phosphorus content of ether extract (dry feces). *Calcium:*[15] To 150 c.c. of urine acetic acid was added. The solution was heated on a water bath for two hours. Any flocculent precipitate produced by this treatment was filtered off and washed with hot water. To the filtrate and washings 10 c.c. of ammonia were added and the mixture was then made slightly acid with acetic. To the acid liquid 15 c.c. of a saturated solution of ammonium oxalate were added and the mixture set aside in a warm place for at least four hours. The precipitate was then filtered off and washed free from oxalate with hot water. The calcium oxalate was dissolved from the paper with hot hydrochloric acid (sp. gr. 1.04), the paper was washed thoroughly with hot water, the solution and washings made up to 150 c.c., heated to 70° C. and titrated with $\frac{n}{20}$ potassium permanganate. The preliminary treatment with acetic acid seems to be necessary only after the urine has stood for a considerable length of time.

Bone ash. — The bone ash employed was a thoroughly incinerated carbon free commercial product. A large quantity was put through a fine sieve and preserved for the experiments in a glass stoppered bottle. In each of the metabolism experiments the amount of bone ash ordinarily given to animals under observation here, namely, 1 gm. per kilo of body weight, was employed. Analysis of the stock supply of bone ash gave the following data for calcium, magnesium, phosphate, sulphate, and residue insoluble in dilute HCl (1:5).

Ca	39.020 per cent	P_2O_5	40.650 per cent
Mg	0.486 per cent	SO_4	0.500 per cent
Insoluble residue	0.089 per cent		

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[13] Gill and Grindley: Journal of the American Chemical Society, 1909, xxxi, p. 52.

[14] Folin: Journal of biological chemistry, 1905, i, p. 154, and This journal, 1905, xiii, p. 52.

[15] Steel and Gies: This journal, 1907, xx, p. 351.

2. **First metabolism experiment.**— A black and tan male dog weighing 9.56 kilos, was given daily 150 gm. of meat, 40 gm. of cracker meal, 30 gm. of lard, and 350 c.c. of water. The preparatory period was six days in length. Elimination of black, medium hard, foul-smelling stools occurred on the second and sixth days. On the eighth to fourteenth days inclusive, 10 gm.

TABLE I.

FIRST EXPERIMENT. DAILY RECORDS.

I. Fore period, Normal conditions.

Number of the day . . .	1	2	3	4	5	6	7	Av.
Body weight (kilos) . . .	9.56	9.61	9.46	9.46	9.45	9.38	9.38	....
Urine: volume (c.c.) . . .	375	295	535	375	410	410	380	397
Sulphur of ethereal sulphates (gm.)	0.1608	0.0705	0.1039	0.1019	0.1030	0.0878	0.0822	0.1014
Feces: dry weight (gm.) .	0	22.5	0	0	0	19	0	5.93

II. Dosage period. 10 gm. of bone ash per diem.

Number of the day . . .	8	9	10	11	12	13	14	Av.
Body weight (kilos) . . .	9.44	9.33	9.31	9.30	9.32	9.25	9.23	....
Urine: volume (c.c.) . . .	335	370	360	350	330	380	380	358
Sulphur of ethereal sulphates (gm.)	0.0809	0.0712	0.0688	0.0648	0.0580	0.0533	0.0681	0.0664
Feces: dry weight (gm.) .	0	41	17.5	11	23	23	21	19.5

III. After period. Normal conditions.

Number of the day . . .	15	16	17	18	19	20	21	Av.
Body weight (kilos) . . .	9.24	9.25	9.21	9.25	9.28	9.27	9.28	....
Urine: volume (c.c.) . . .	360	375	330	325	345	420	425	368
Sulphur of ethereal sulphates (gm.)	0.0476	0.0549	0.0582	0.0570	0.0506	0.0612	0.0501	0.0542
Feces: dry weight (gm.) .	0	0	12	0	0	0	16.5	4.07

of bone ash were mixed with the daily food. On the ninth day there was a large amount of fecal matter, white and hard and free from odor. Defecation occurred daily throughout the rest of the dosage period. During the *after* period there was elimination, on the seventeenth and twenty-first days, of soft feces like those of the fore period. The dog showed absolutely no visible effects of the bone ash. The weight during the experiment fell to 9.28 kilos, doubtless because the prescribed diet was insufficient in amount. The reaction of the daily urine was uniformly acid to phenolthalin, amphoteric to litmus, and alkaline to rosolic acid. The specific gravity of the urine fluctuated between 1013 (twentieth day) and 1018 (seventeenth and eighteenth days).

The analytical data of this experiment are summarized in Tables I, II, and III.

TABLE II.

FIRST EXPERIMENT. URINE. ANALYTICAL TOTALS AND DAILY AVERAGES FOR EACH PERIOD.

Period (7 d'ys).	Volume.		Ash.		Nitrogen.		Sulphur.		P_2O_5.		CaO.	
	T'al.	D'y av.	Total.	D'ly av.	T'al.	D'y av.	Total.	Daily av.	T'al.	Daily av.	Total.	Daily av.
	c.c.	c.c.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Fore .	2862	397	13.216	1.888	45.40	6.48	2.3835	0.3405	5.953	0.8505	0.1834	0.0262
Dosage	2505	358	11.564	1.652	43.09	6.16	2.2911	0.3273	3.824	0.5463	0.1099	0.0157
After .	2580	368	12.320	1.760	45.40	6.48	2.6418	0.3774	5.355	0.7651	0.1540	0.0220

TABLE III.

FIRST EXPERIMENT. FECES. ANALYTICAL TOTALS AND DAILY AVERAGES FOR EACH PERIOD.

Period (7 days).	Weight.		Nitrogen.		Ash.		Fat.		Lecithin.	
	Total.	Daily av.	Total.	Daily av.	Total.	Daily av.	Total.	Daily av.	Total.	Daily av.
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Fore . . .	41.5	5.93	2.482	0.354	4.316	0.616	6.80	0.971	0.112	0.016
Dosage . .	136.5	19.50	4.518	0.647	74.530	10.650	7.07	1.010	0.105	0.015
After . . .	28.5	4.07	1.810	0.258	4.090	0.584	3.01	0.430	0.049	0.007

3. **Second metabolism experiment.** — A female Scotch terrier was used in the second experiment and received a daily diet of 135 gm. of meat, 36 gm. of cracker meal, 27 gm. of lard, and 315 c.c. of

TABLE IV.

SECOND EXPERIMENT. DAILY RECORDS.

I. Fore period. Normal conditions.

Number of the day	1	2	3	4	5	6	Av.
Body weight (kilos)	8.95	8.97	8.97	8.98	8.96	8.93	....
Urine: volume (c.c.)	465	350	365	365	360	420	387
Sulphur of ethereal sulphates (gm.)	0.0415	0.0340	0.0334	0.0292	0.0401	0.0194	0.0329
Feces: dry weight (gm.)	0	0	9.0	0	3.5	4.5	2.83

II. Dosage period. 9 gm. of bone ash per diem.

Number of the day	7	8	9	10	11	12	Av.
Body weight (kilos)	8.94	8.96	8.92	8.93	8.89	8.90	....
Urine: volume (c.c.)	335	335	375	357	350	355	351
Sulphur of ethereal sulphates (gm.)	0.0165	0.0165	0.0251	0.0134	0.0194	0.0141	0.0178
Feces: dry weight (gm.)	8	11	17.5	8.5	26	8.5	13.25

III. After period. Normal conditions.

Number of the day	13	14	15	16	17	18	Av.
Body weight (kilos)	8.84	8.84	8.82	8.89	8.92	8.93	....
Urine: volume (c.c.)	320	398	305	260	355	325	327
Sulphur of ethereal sulphates (gm.)	0.0115	0.0232	0.0162	0.0063	0.0181	*	0.0151
Feces: dry weight (gm.)	18	0	10	3	0	11	7

* The urine was lost by accident after it had been made up to 1000 c.c., so that the after period in the quantitative records was shortened to five days.

water. Five days sufficed to accustom the animal to the cage. The weight remained fairly constant throughout the experiment, being 8.95 kilos at the start and 8.93 kilos at the finish. In the *fore* period of six days defecation of black, foul-smelling stools occurred on the third, fifth, and sixth days, and the effect of the bone ash appeared immediately in the feces on the day after the first dose. During the *dosage* period there was daily defecation. The discontinuance of the bone ash seemed to have a disturbing effect, for on the first day of the *after* period there were two diarrheal movements, and on the third and fourth days the feces were still soft, and contained much mucous matter. The reaction of the daily urine was always acid to phenolthalin and alkaline to rosolic acid. It was acid to litmus on all but the last five days

TABLE V.

SECOND EXPERIMENT. URINE. ANALYTICAL TOTALS AND DAILY AVERAGES FOR EACH PERIOD.

Period.	Volume.		Ash.		Nitrogen.		Sulphur.		P_2O_5.		CaO.	
	T'al.	D'y av.	T'al.	D'ly av.	T'al.	D'l av.	Total.	Daily av.	T'al.	Daily av.	Total.	Daily av.
	c.c.	c.c.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Fore . .	2325	387	10.32	1.720	36.42	6.07	1.7958	0.2993	4.49	0.7485	0.0918	0.0153
Dosage .	2107	351	10.68	1.780	36.06	6.01	1.9704	0.3284	4.04	0.6732	0.0792	0.0132
After . .	1963	327	7.84	1.568	30.20	6.04	1.5350	0.3070	3.68	0.7370	0.0785	0.0157

TABLE VI.

SECOND EXPERIMENT. FECES. ANALYTICAL TOTALS AND DAILY AVERAGES FOR EACH PERIOD.

Period (6 days).	Weight.		Nitrogen.		Ash.		Fat.		Lecithin.	
	Total.	Daily av.	Total.	Daily av.	Total.	Daily av.	Total.	Daily av.	Total.	Daily av.
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Fore . . .	17.0	2.83	1.183	0.197	2.295	0.38	2.96	0.493	0.036	0.006
Dosage . .	79.5	13.25	2.258	0.376	48.020	8.00	2.86	0.477	0.024	0.004
After . . .	42.0	7.00	2.065	0.344	12.410	2.07	4.12	0.687	0.018	0.003

of the after period, when it was amphoteric to that indicator. The specific gravity of the urine fluctuated between 1015 and 1018. During the experiment the animal was pregnant, and two sturdy pups were born a month after the close of the experiment. The bone ash had no discernible effects on the mother or the pups.

The daily records and analytical data of the second metabolism experiment are summarized in Tables IV–VI.

4. **Third metabolism experiment.** — The animal, a small shaggy black and tan male dog, weighing 6.90 kilos, was given a daily diet consisting of 105 gm. of meat, 28 gm. of cracker meal, 21 gm. of lard, and 245 c.c. of water. The dog was under preliminary observation in a cage for twelve days, during which there was apparently no normal defecation. It was suspected, however, that the dog had been defecating but that he was disposing of the fecal matter, for on the fifth day a very small amount of feces (½ gm.) was found on the pan. Accordingly, the animal was carefully watched. Meanwhile the daily records and excreta were kept. On the twelfth day a small amount of hard, black, and doughy material was passed. The dosage period was thereupon begun, and the urine samples of that and the five preceding days were taken to represent the fore period. Seven grams of bone ash were mixed with the food of the seventh day, and the characteristic bone ash feces appeared within twenty-four hours. The animal was kept under continuous observation during the daytime, and on the tenth day, about an hour after feeding, defecation occurred and it was noticed that the animal was licking his chops as if he had disposed of a portion of the feces. Although bone ash had been given on three successive days, the fecal matter was dark-colored and foul, and a small amount was purposely left on the wire to test the dog. It was soon ingested. On the following day fecal crumbs on the pan showed evidence of defecation, but there was no material on the wire. On the three succeeding days the excrements were dry and very hard, and were avoided by the animal. The dosage period was continued for eight days, and an after period of seven days followed, defecation occurring on the first, third, and last day of the period.

In spite of the fact that there was evident ingestion of some of the feces, the animal did not seem to exhibit any symptoms whatever. The output of ethereal sulphates showed an increasing amount of intestinal putrefaction up to the time when the fecal matter became hard and dry and was avoided by the dog. However, the putrefactive products seemed to produce no toxic effect,

TABLE VII.

THIRD EXPERIMENT. DAILY RECORDS.

I. Fore period. Normal conditions.

Number of the day	1	2	3	4	5	6	Av.
Body weight (kilos)	6.74	6.75	6.81	6.74	6.74	6.81	...
Urine: volume (c.c.)	260	230	170	310	215	180	227
Sulphur of ethereal sulphates (gm.)	0.0253	0.0253	0.0217	0.030	0.0263	0.0339	0.0271
Feces: dry weight (gm.)	0	0	0	0	0	9.0	1.5

II. Dosage period. 7 gm. of bone ash per diem.

Number of the day	7	8	9	10	11	12	13	14	Av.
Body weight (kilos)	6.87	6.92	6.96	6.97	6.95	6.97	6.92	6.90	...
Urine: volume (c.c.)	150	190	170	215	200	190	225	240	201
Sulphur of ethereal sulphates (gm.)	0.0274	0.0369	0.0306	0.0438	0.0552	0.0416	0.046	0.0425	0.0405
Feces: dry wt. (gm.)	0	7	0	14.5	0	17.5	15.0	11.5	8.2

III. After period. Normal conditions.

Number of the day	15	16	17	18	19	20	21	Av.
Body weight (kilos)	6.81	6.84	6.85	6.88	6.88	6.91	6.91	...
Urine: volume (c.c.)	235	215	220	200	240	215	235	223
Sulphur of ethereal sulphates (gm.)	0.0386	0.0288	0.0247	0.0328	0.0412	0.0325	0.0318	0.0329
Feces: dry weight (gm.)	14.0	0	3.5	0	0	0	3.5	3

for the animal was very lively and apparently normal throughout the experiment and never became at all lethargic.

The reaction of the daily urine was uniformly acid to phenolthalin, amphoteric to litmus, and alkaline to rosolic acid. The specific gravity of the urine fluctuated between 1026 (seventh day) and 1015 (twenty-first day); it was usually above 1016 and below 1022.

The daily records and analytical data of the third metabolism experiment are summarized in Tables VII–IX.

TABLE VIII.

Third Experiment. Urine. Analytical Totals and Daily Averages for each Period.

Period.	Volume.		Ash.		Nitrogen.		Sulphur.		P_2O_5.		CaO.	
	T'al.	D'y av.	T'al.	D'ly av.	T'al.	D'y av.	Total.	Daily av.	T'al.	Daily av.	Total.	Daily av.
	c.c.	c.c.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Fore .	1365	227	7.584	1.264	26.72	4.45	1.5222	0.2537	3.190	0.5317	0.1152	0.0192
Dosage.	1610	201	9.888	1.236	33.18	4.14	2.4500	0.3064	4.495	0.5619	0.2512	0.0314
After .	1560	223	8.568	1.224	29.42	4.20	1.6569	0.2367	3.713	0.5305	0.1596	0.0228

TABLE IX.

Third Experiment. Feces. Analytical Totals and Daily Averages for each Period.

Period.	Weight.		Nitrogen.		Ash.		Fat.	
	Total.	Daily av.	Total.	Daily av.	Total.	Daily av.	Total.	Daily av.
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Fore	9.0	1.50	0.473	0.0788	9.85	0.164	1.70	0.283
Dosage	65.5	8.20	1.520	0.1900	37.27	4.660	3.12	0.390
After	21.0	3.00	0.619	0.0884	11.13	1.576	0.93	0.133

5. **Fourth metabolism experiment.** — The animal chosen for this experiment was a young dog of a white mongrel type. The daily diet was as follows: 180 gm. of meat, 48 gm. of cracker meal, 36 gm. of lard, and 420 c.c. of water. When the dog was first confined in the cage, his weight was 11.72 kilos, and during the first week it increased about 50 gm. per day, so that at the end of seven days it had risen to 12.12 kilos. During the subsequent week he continued to gain in weight, and it was evident that he was a growing dog. It was finally decided to start the experiment on this

ascending scale of weight. The dog continued to increase from 12.37 kilos at the beginning of the experiment to 12.91 kilos at the end.

During a *fore* period of four days defecation occurred daily, and the feces were black, diarrheal, and contained much mucous matter. On the fifth day 12 gm. of bone ash were mixed with the food. The feces on the two following days were still soft but yellow and

TABLE X.

Fourth Experiment. Daily Records.

I. Fore period. Normal conditions.

Number of the day	1	2	3	4	Average.
Body weight (kilos)	12.37	12.34	12.47	12.47	...
Urine: volume (c.c.)	300	320	250	280	287
Sulphur of ethereal sulphates (gm.)	0.0329	0.0277	0.0193	0.0346	0.0286
Feces: dry weight (gm.)	7	8.5	2	12	7.4

II. Dosage period. 12 gm. of bone ash per diem.

Number of the day	5	6	7	8	9	10	Av.
Body weight (kilos)	12.50	12.52	12.57	12.62	12.66	12.71	...
Urine: volume (c.c.)	275	295	320	270	260	275	283
Sulphur of ethereal sulphates (gm.)	0.0255	0.0191	0.0339	0.0379	0.0307	0.0272	0.0280
Feces: dry weight (gm.)	18	19	9	17	16.5	20	16.6

III. After period. Normal conditions.

Number of the day	11	12	13	14	15	Av.
Body weight (kilos)	12.77	12.82	12.84	12.87	12.91	...
Urine: volume (c.c.)	300	320	330	360	300	322
Sulphur of ethereal sulphates (gm.)	0.0263	0.0255	0.0211	0.0289	0.0293	0.0262
Feces: dry weight (gm.)	13.5	0	10	0	4.5	5.6

free from mucus. On the seventh day the feces were formed, and on the ninth day they were hard and dry. Bone ash was administered for six days. During the *after* period of five days defecation occurred on alternate days, and on the thirteenth day the feces were again black and free from bone ash, but did not show the diarrheal characteristics of the feces of the *fore* period.

TABLE XI.

FOURTH EXPERIMENT. URINE. ANALYTICAL TOTALS AND DAILY AVERAGES FOR EACH PERIOD.

Period.	Volume.		Ash.		Nitrogen.		Sulphur.		P_2O_5.		CaO.	
	T'al.	D'y av.	T'l.	D'y av.	T'al.	D'y av.	Total.	Daily av.	T'l.	Daily av.	Total.	Daily av.
	c.c.	c.c.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Fore . . .	1150	287	5.68	1.42	22.94	5.73	1.2652	0.3163	3.09	0.7727	0.0680	0.0170
Dosage . .	1695	283	8.93	1.49	33.91	5.65	1.9074	0.3179	4.06	0.6771	0.1152	0.0192
After . . .	1610	322	7.16	1.44	30.06	6.01	1.7655	0.3531	3.47	0.6949	0.0720	0.0144

TABLE XII.

FOURTH EXPERIMENT. FECES. ANALYTICAL TOTALS AND DAILY AVERAGES FOR EACH PERIOD.

Period.	Weight.		Nitrogen.		Ash.		Fat.	
	Total.	Daily av.	Total.	Daily av.	Total.	Daily av.	Total.	Daily av.
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Fore	29.5	7.4	2.407	0.602	2.64	0.66	3.62	0.905
Dosage	99.5	16.6	3.283	0.547	58.01	9.66	5.15	0.858
After	28.0	5.6	1.540	0.308	8.87	1.77	3.05	0.610

The great utility of bone ash in preventing diarrhea was strikingly shown in this animal. The discharges at first were very watery, and twice the experiment had to be begun anew on account of the mingling of urine and feces. The first dose of bone ash had a marked effect, and on the third day the diarrhea was completely stopped.

The reaction of the daily urine was uniformly acid to phenolthalin, amphoteric to litmus, and alkaline to rosolic acid. The specific gravity of the urine fluctuated between 1017 (twelfth and fourteenth days) and 1021 (first day).

The daily records and summaries of the fourth metabolism experiment are given in Tables X–XII.

6. **General comparison of the results of the four metabolism experiments.** — A study of the summaries in Tables XIII and XIV, together with the data in Tables I and XII inclusive, warrants the following deductions:

Nitrogen balance. — In the *first* experiment the slight negative balance is probably accounted for by the fact that the prescribed diet, as mentioned on page 304, was not sufficient for that particular animal. There was, therefore, a utilization of a small amount of body protein with a decrease in weight during the experiment. The weight was very constant during the after period of the first experiment, when nitrogenous equilibrium was practically established. In the *fourth* experiment there was a steady increase in weight throughout the work, and the excessive plus balances show that a relatively large amount of nitrogen was utilized in building up body tissue. The *third* animal gained somewhat in body weight; slightly more in the dosage than in the other periods. The positive balances in the different periods accord with that observation. With the *second* dog the weight was practically constant throughout the experiment (page 306), although there was a continuous negative balance.

It is obvious that the administration of bone ash had no particular effect in these experiments on the total nitrogenous metabolism.

Urine volume. — In all the experiments there was a *decrease* in urine volume during the periods of the administration of bone ash, probably because of greater elimination of water in the increased bulk of fecal matter. In all but one case the average daily volume in the after period was greater than that in the corresponding dosage period. In only one instance was the average volume in the after period greater than that in the corresponding fore period. In the second animal the discontinuance of the dosage with bone ash was coincident with a tendency to diarrheal movements and a consequent diminution in the daily average urine volume for the after period. The large increase exhibited by the fourth animal in the after period was probably due to the fact that he was a growing dog, and, as he continued to gain in weight, less water was utilized

TABLE XIII.

SUMMARY OF COMPARATIVE DAILY AVERAGES OF THE ANALYTICAL DATA, PER PERIOD, OF THE FOUR METABOLISM EXPERIMENTS.

	Urine (volume in c.c.).					Feces (weight in gm.).				
Period.	1	2	3	4	Gen. av.	1	2	3	4	Gen. av.
Fore . . .	397	387	227	287	324	5.93	2.83	1.50	7.38	4.41
Dosage . .	358	351	201	283	298	19.50	13.25	8.20	16.58	14.38
After . . .	368	327	223	322	310	4.07	7.00	3.00	5.60	4.92
	Nitrogen.					Nitrogen.				
Fore . . .	6.48	6.07	4.45	5.74	5.69	0.354	0.197	0.079	0.602	0.308
Dosage . .	6.16	6.01	4.14	5.65	5.49	0.647	0.376	0.190	0.547	0.440
After . . .	6.48	6.04	4.20	6.01	5.68	0.258	0.344	0.088	0.308	0.249
	Ash.					Ash.				
Fore . . .	1.88	1.72	1.26	1.42	1.57	0.616	0.382	0.164	0.660	0.455
Dosage . .	1.65	1.78	1.23	1.49	1.53	10.650	8.000	4.660	9.660	8.240
After . . .	1.76	1.57	1.22	1.44	1.49	0.584	2.070	1.580	1.770	1.500
	Sulphur.					Fat (ether extract).				
Fore . . .	0.340	0.299	0.254	0.316	0.302	0.971	0.493	0.283	0.905	0.663
Dosage . .	0.327	0.328	0.306	0.318	0.319	1.010	0.477	0.390	0.858	0.684
After . . .	0.377	0.307	0.237	0.353	0.318	0.430	0.687	0.133	0.610	0.465
	CaO.					Lecithin (ether extract).				
Fore . . .	0.026	0.015	0.019	0.017	0.019	0.016	0.006	. . .	. . .	. . .
Dosage . .	0.016	0.013	0.031	0.019	0.020	0.015	0.004	. . .	. . .	. . .
After . . .	0.022	0.016	0.023	0.014	0.019	0.007	0.003	. . .	. .	. . .
	P_2O_5.					Urine. Sulphur of ethereal sulphates.				
Fore . . .	0.850	0.743	0.532	0.773	0.726	0.101	0.033	0.027	0.029	0.047
Dosage	0.546	0.673	0.562	0.677	0.614	0.066	0.018	0.040	0.028	0.038
After . . .	0.765	0.738	0.530	0.695	0.682	0.054	0.015	0.033	0.026	0.032

than at the start. Although receiving 420 c.c. daily, the largest volume eliminated on any one day was 360 c.c., — on the day previous to the close of the experiment.

Ash. — The amount of ash that was excreted in the urine was practically constant; in the last two experiments it was almost identical for each period. This fact indicates that there was no appreciable absorption of the inorganic constituents of the administered bone ash, although such an absorption might occur without any corresponding increase in the amount of urinary ash. In two cases there was a decrease and in the two others a slight increase of urinary ash during the dosage period. The amount of fecal ash was, of course, very much greater for the periods during which bone ash was given. The large amount of fecal ash for the after period in three of the four experiments showed that considerable bone ash persisted in the intestinal tract and was not completely eliminated for several days. With the first animal practically complete elimination of ingested bone ash occurred within twenty-four hours after the interruption of the treatment with bone ash. The daily average amount of fecal ash in the corresponding *after* period was 0.58 gm., practically the same as that of the *fore* period, — 0.62 gm.

Nitrogen. — In each case there was a *slight* decrease in urinary

TABLE XIV.

NITROGEN BALANCE IN EACH PERIOD OF THE FOUR METABOLISM EXPERIMENTS.

Experiment.	1			2			3			4		
Period.	Fore. (7 days.)	Dosage. (7 days.)	After. (7 days.)	Fore. (6 days.)	Dosage. (6 days.)	After. (5 days.)	Fore. (6 days.)	Dosage. (8 days.)	After. (7 days.)	Fore. (4 days).	Dosage. (6 days.)	After. (5 days.)
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Food	46.97	46.97	46.97	36.23	36.23	30.20	29.50	39.36	34.44	33.72	50.58	42.15
Excreta	47.84	47.63	47.17	37.60	38.32	32.26	27.17	34.64	30.00	25.34	37.18	31.60
Balance	−0.87	−0.66	−0.20	−1.37	−2.09	−2.06	+2.33	+4.72	+4.44	+8.38	+13.40	+10.55

nitrogen, and in three cases corresponding increases in fecal nitrogen, during the bone ash periods. Possibly there was *slightly* less assimilation of nitrogenous material during the dosage period, and probably, also, at the same time an entanglement of a *slightly* larger amount of mucous matter by the increased bulk of excrementitious material. The urinary nitrogen in each after period was always greater than that of the corresponding dosage period. The fourth dog, which was not in weight equilibrium, but utilizing nitrogenous matter in building up body tissue, did not exhibit the increase of fecal nitrogen shown by the other animals during the dosage periods. The high content of fecal nitrogen for this dog in the fore period confirmed the observation of the presence of much mucous matter in the diarrheal stools mentioned on page 310, the same cause accounting for the high figure in the feces of the after period of the experiment on the second dog (page 306).

Sulphur. — (*a*) Ethereal sulphates. In the first two experiments there was a constant decrease in the output of ethereal sulphates. A minimum was reached during the latter part of the dosage period, showing that bone ash, probably by increasing the bulk of the feces and so causing daily evacuation, kept down intestinal putrefaction. In the third experiment, where there was evidence of the ingestion of fecal matter, the amounts of ethereal sulphates rose gradually until the middle of the dosage period, when the feces became hard and dry and were avoided by the animal as mentioned on p. 307. Then, as *daily* defecation was inaugurated and no feces were ingested, the quantitative output of ethereal sulphate gradually decreased again. In the fourth experiment the quantitative elimination was practically the same in each period. (*b*) Total sulphur. The amount of total sulphur showed no consistent regularity. In two experiments (II and III) it was highest during the dosage periods, and in the other two (I and IV) in the after periods. Probably the increase in these two periods was largely dependent on the elimination of the sulphur in the bone ash (page 302).

Calcium. — The variations in the output of urinary calcium were hardly more than the normal fluctuations. The quantity in dog's urine is small at best, and shows no noticeable increase during the ingestion of bone ash, notwithstanding the relatively large amount of calcium present in bone ash. In two cases there were slight decreases and in two slight increases of calcium output in the urine during the dosage periods. In the third experiment there was a perceptible increase not only in urinary calcium excretion, but also in the amount of urinary phosphorus during the dosage period. Probably the ingestion of the fecal material already noted (page 307) brought about conditions which allowed a somewhat greater absorption of calcium phosphate. In that experiment (III) the amount of urinary sulphur was also much higher.

Phosphorus. — Except in the instance already mentioned in the discussion of the calcium content, there was in each experiment a marked decrease in urinary phosphorus during the dosage period with a subsequent increase during the after period, the latter amounts, however, in no case quite reaching the high mark of the fore period. The third experiment shows considerable deviation from the other three. The results prove without doubt that there was no appreciable absorption of the calcium phosphate of the bone ash. The decrease in phosphorus output in the urine may be accounted for, as suggested by Steel and Gies, by the probability of a diminished absorption of alkali phosphate from the food. "Such a result might ensue from interaction, in the intestine particularly, between calcium and phosphate with the production of less soluble or precipitated products. Possibly such interaction would occur especially between alkali phosphate and that portion of the available calcium that had been converted into chlorid from carbonate, and which chlorid would be prone, in the intestine, to combine with phosphate in increasing proportion as the mixture containing them became less acid or perhaps alkaline in reaction. . . . The *total* amount of phosphorus (phosphate) in the excreta would doubtless be unaffected, for the phosphate withheld from absorption would be passed, within a few hours, as calcium phosphate into the feces."[16]

Fat absorption. — The amounts of unabsorbed fat in the feces indicated only a normal variation. The differences were in no case great enough to warrant the statement that the bone ash exercised any influence one way or the other upon the absorption of fats. In each of two experiments there was a slight increase and in the other two slight decreases in the amounts excreted. In three cases there was a more perceptible decrease during the after periods, suggesting, perhaps, a somewhat more complete assimilation, but not more than that of half a gram at the most out of 30 gm. given in the diet.

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[16] STEEL and GIES: This journal, 1907, xx, p. 351.

Fecal lecithin. — It seemed desirable to determine the approximate amount of lecithin in the fecal fatty matter in order to obtain, if possible, a suggestion regarding the effect of bone ash on the secretion of bile. Phosphorus in the residue was determined, and the amount of lecithin calculated therefrom with the aid of the figure for the phosphorus content of distearyl lecithin: 3.57 per cent. The results showed that there was no effect of the bone ash on the elimination of lecithin and probably none on the secretion of bile.

III. ARTIFICIAL DIGESTION EXPERIMENTS.

Although the experiments described on the foregoing pages made it seem quite improbable that bone ash affects unfavorably either the digestive or absorptive processes, the following experiments were performed in order to reveal any possible effects on the activity of the gastro-intestinal enzymes.

1. **Salivary digestion.** — Two cubic centimetres of saliva immediately digested the starch in 18 c.c. of 1 per cent paste containing as much as 0.4 gm. of bone ash. Preliminary tests with saliva greatly diluted with water to prevent rapid digestion of starch made it evident that ptyalin is markedly restrained by comparatively large proportions of bone ash. The following data are among the most significant that were obtained in this connection:

A. Conditions: Starch paste, 1 per cent — 18 c.c. Saliva, 1 in 5 — 2 c.c.

No.	1	2	3	4	5	6
Bone ash (gm.)	0	0.025	0.05	0.1	0.2	0.4
Achromic point attained in	4 min.	6 min.	10 min.	Red after 1½ hrs.		Purple after 1½ hrs.

B. Conditions: Starch paste, 1 per cent — 13 c.c. Saliva, 1 in 50 — 2 c.c.

No.	1	2	3	4	5	6
Bone ash (gm.)	0	0.025	0.05	0.1	0.2	0.3
Achromic point attained in	1 hr., 10 min.	11 hrs.	24 hrs.	Blue, 24 hrs. later		

C. Conditions: Starch paste, 1 per cent — 18 c.c. Saliva, 1 in 20 — 2 c.c.

No.	1	2	3	4	5	6
Bone ash (gm.)	0	0.005	0.01	0.02	0.05	0.1
Achromic point attained in	20 min.	20 min.	35 min.	35 min.	36 hrs.	42 hrs.

Slight quantities of soluble calcium salts, such as calcium chloride, seem to have beneficial effects in such experiments, but tribasic calcium phosphate was found to behave like bone ash. Calcium carbonate, while not so effective as tribasic calcium phosphate, retarded markedly the action of the ptyalin in similar experiments under the same conditions.

Analogous experiments with solutions of diastase gave similar results.

D. Conditions: Starch paste, 1 per cent — 18 c.c. Diastase sol., 0.1 per cent — 2 c.c
(Digestion started at 5 P. M.)

No.	1	2	3	4	5	6
Bone ash (gm.)	0	0.01	0.02	0.5	0.1	0.5
Achromic point attained in	3 hrs.	Complete at 9 A. M.	Red at 9 A. M.	Purple at 9 A. M.	Still blue at 9 A. M.	

E. Conditions: Starch paste, 1 per cent — 15 c.c. Diastase sol., 0.1 per cent — 5 c.c.

No.	1	2	3	4	5
Bone ash (gm.)	0	0.01	0.02	0.05	0.1
Achromic point attained in	1½ hrs.	2 hrs.	2¼ hrs.	4 hrs.	Red 24 hrs. later.

Other insoluble substances, such as glass wool, cotton, sand, infusorial earth, and barium sulphate, were tried under the same conditions, but none of them hindered the action of either ptyalin or diastase. The effect of bone ash seemed to be due to the insoluble calcium salts contained in it.

The water soluble material from 0.5 gm. of bone ash was obtained by shaking repeatedly with 20 c.c. of water. After filtration, 10 c.c. of the extract were mixed with an equal volume of starch paste, and 2 c.c. of diluted saliva (1:5) were added. The digestion was not appreciably affected. This seems to show that the interference of the bone ash is mechanical rather than chemical.

2. **Peptic digestion.** — Experiments with pepsin-HCl were carried out, in general, according to the method described by Berg and Gies,[17] a brief outline of which follows:

> Weighed amounts of bone ash were transferred to wide-mouthed glass-stoppered bottles having a capacity of about 150 c.c. One hundred cubic centimetres of a solution containing 0.2 per cent of hydrochloric acid and 0.1 per cent or 0.3 per cent of pepsin ("Merck, Ph. G. IV") were then added to each bottle, followed by 1 gm. of fibrin or elastin, which had been dried to constant weight at 110° C. The bottles containing the mixtures

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[17] Berg and Gies: Journal of biological chemistry, 1907, ii, p. 497.

were then placed in a water bath maintained at 40° C. throughout the experiment. Controls were also run: protein alone, and bone ash alone, in comparable volumes of the pepsin-acid solution. Each mixture in a series was subjected, of course, to the same general conditions as all the others.

At the conclusion of the digestive period the mixtures were filtered on dry weighed papers which were subsequently desiccated and weighed, giving, by difference, the amount of undigested protein plus any undissolved bone ash. The amount of undissolved ash in the corresponding control subtracted from the total weight of undissolved protein and ash gave the amount of protein residue (undigested matter).

In the tests with fibrin aliquot portions of the filtrates were used for the quantitative determination of the metaprotein.[18] To stop digestion at a definite time after filtration was started, the liquid was made alkaline with dilute potassium hydroxide solution. Finally, while hot, the alkaline filtrates were neutralized and then made very faintly acid to lacmoid with dilute hydrochloric acid, in order to keep in solution the calcium phosphate from the bone ash and also to favor the complete precipitation of the metaprotein. After standing over night the solutions were filtered through weighed papers, the precipitates washed free from saline matters, and the papers dried and weighed. The combined weight of the neutralization precipitate (per 100 c.c.) plus that of the protein residue subtracted from 1 gm. gave the weight of combined fibrin proteoses and peptones. In the case of elastin no precipitable metaprotein is formed in peptic digestion, so that the weight of the protein residue subtracted from 1 gm. gave the weight of the corresponding elastin proteoses and peptones.

Observations of the digestive process in these experiments indicated that with increase in the amount of bone ash there were also larger amounts of protein residue, due, possibly, merely to the decrease in the amount of hydrochloric acid by the neutralizing effect of the bone ash. The quantitative data show that in 100 c.c. of the pepsin-acid mixtures, containing not more than 300 mg. of bone ash (which amounts were completely dissolved in the control), digestion of fibrin was not seriously interrupted and the amounts of combined proteoses and peptones that were formed did not vary appreciably. Larger amounts of bone ash, however, by greatly reducing the acidity, appreciably retarded the peptic digestive process in the case of fibrin, as was shown by the correspondingly larger amounts of residue and by the production of much less material beyond the metaprotein stage. Even larger quantities of bone ash were required to appreciably affect the peptic digestion of elastin.

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[18] Hawk and Gies: This journal, 1902, vii, p. 460; also Gies and collaborators: Biochemical researches, 1903, i, p. 615.

Typical data in this connection are summarized below:

A. *Elastin.* 1 gm. 100 c.c. of 0.2 per cent HCl — 0.3 per cent pepsin solution. Digestive period: 10 hours.

No.	1	2	3	4	5	6
Bone ash (mg.)	0	100	200	300	500	700
Residue (mg.)	849	861	869	862	870	903

B. *Fibrin* I. 1 gm. 100 c.c. of 0.2 per cent HCl — 0.1 per cent pepsin solution. Digestive period: 1¾ hours.

No.	1	2	3	4	5
Bone ash (mg.)	0	100	200	300	500
Residue (mg.)	25	33	31	55	127
Neutralization precipitate (mg.)	264	255	206	243	303
Combined proteoses and peptones (mg.)	711	712	763	702	570

C. *Fibrin* II. 1 gm. 100 c.c. of 0.2 per cent HCl—0.1 per cent pepsin solution. Digestive period: 1¾ hours.

No.	1	2	3	4	5	6
Bone ash (mg.)	0	100	200	300	400	700
Residue (mg.)	72	84	96	127	248	315
Neutralization precipitate (mg.)	149	186	158	206	229	301
Combined proteoses and peptones (mg.)	779	730	746	667	523	384

Twenty milligrams of pepsin, dissolved in about 2 c.c. of water, were added to 5 gm. of bone ash and the thin paste mixed at intervals for about four hours. The mixture was filtered and 1 c.c. added to 20 c.c. of 0.2 per cent HCl. A small piece of fibrin, kept in the mixture at 40° C. for an hour, was unaffected. Evidently the alkalinity of the bone ash was sufficient to destroy the pepsin in the comparatively concentrated solution employed. In the stomach, however, there is normally sufficient acid to immediately neutralize the slight alkalinity of the bone ash, in amounts even greater than those of the doses given in the metabolism experiments.

The effect of bone ash on specially *dilute* pepsin solution was determined under the following conditions: 20 c.c. of 0.2 per cent HCl containing 0.01 per cent of pepsin (Merck, "Ph. G. IV"); fibrin, 0.1 gm.; temperature, 40° C. The experiment was started at 9 P. M.

No.	1	2	3	4
Bone ash (mg.)	0	50	100	200

After three hours digestion was evident in 1 and 2; it was practically complete in 1 and 2 at nine o'clock on the following morning. In each of 3 and 4 there was an appreciable residue, even at the end of twenty-four hours, but after standing for several days digestion in 3 and 4 was also practically complete, although free acid was absent from 4.

3. **Tryptic digestion.** —The general method of procedure in the tryptic experiments was the same as in the peptic digestions. A solution containing 0.25 per cent of Na_2CO_3 and 0.1 per cent of trypsin (Merck) was used, with fibrin or elastin as the indicator. The weight of the soluble matter in 1 gm. of bone ash was determined, and from this the corresponding amounts in the various quantities used were calculated. Observations of the digestion as it proceeded suggested that the amounts of residue were practically the same in all the bottles. The quantitative data indicate that artificial tryptic digestion is not materially interfered with by bone ash under the conditions of these experiments. The amounts of elastin residue decreased as the amounts of bone ash were increased in a series, but, in the case of fibrin, there was a gradual rise in the corresponding amounts of metaprotein, and a consequent decrease in the quantities of combined proteoses and peptones. The increase in the amount of metaprotein was gradual and fairly constant, amounting to an increase of 100 mg. in the presence of 2 gm. of the ash. Typical data are subjoined:

A. Fibrin. 1 gm. 100 c.c. of 0.25 per cent Na_2CO_3–0.1 per cent trypsin solution. Digestive period: 2 hours.

No.	1	2	3	4	5	6
Bone ash (mg.)	0	100	200	500	1000	2000
Residue (mg.)	53	66	46	57	56	72
Neutralization precipitate (mg.)	177	191	201	215	271	275
Proteoses and peptones (mg.)	770	743	753	728	673	653

B. Elastin. 1 gm. 100 c.c. of 0.25 per cent Na_2CO_3–0.1 per cent trypsin solution. Digestive period: 1½ hours.

No.		1	2	3	4	5	6
Bone ash (mg.)		0	100	200	500	1000	2000
Residue (mg.)	Exp. 1	136	117	92	78	99	79
	Exp. 2	116	51	45	44	62	71

4. **Pancreatic amylolytic digestion.** — For digestive experiments with amylopsin a glycerol extract of sheep's pancreas was employed. The acidity of the extract, due to acid phosphates, was

neutralized with 0.5 per cent Na_2CO_3, which was then added in excess until the reaction became faintly alkaline to litmus. A 1 per cent starch paste solution was employed, and the observations conducted under conditions similar to those of the salivary digestion experiments (page 317). Ten drops of the extract and 20 c.c. of starch paste were mixed with different amounts of bone ash. The results were very similar to those obtained in the salivary experiments. Small amounts of bone ash had a decided retarding effect, and larger amounts prevented digestion to the achromic point for many hours. The bone ash decidedly interferes with the action of the amylases, seemingly in a mechanical way. The effect on the amylopsin was less marked than that on the ptyalin, probably because of the greater concentration of the former under the prevailing conditions.

5. **Lipolytic digestion.** — A portion of the glycerol pancreatic extract that had been prepared for use in the amylolytic experiments was also employed for the lipolytic experiments. The acid reaction of fresh milk was neutralized with Na_2CO_3, and then the milk was colored strongly blue with litmus solution. Three cubic centimetres of the pancreatic extract were added to 25 c.c. of milk in several bottles containing different amounts of bone ash. The bottles were kept in water at 40° C. and the colors compared from time to time. If under these conditions an extract displays lipolytic power, there is a gradual change from blue to red, caused by the hydrolytic production of fatty acid from milk fats, the intensity of the red coloration indicating the relative speed of the reaction.

It was very difficult to distinguish between the shades of color in the control tubes and those containing 0.1, 0.2, or 0.4 gm. of bone ash. The tubes containing 0.8 and 1.6 gm. showed a distinct bluish color. The tube containing 1.6 gm. remained as blue as the original milk for a long time. On long standing there was a slow change of color in all the samples. The action of the lipase was not appreciably interfered with by moderate amounts of bone ash. It is a question whether the larger amounts of bone ash interfered chemically by neutralizing the fatty acids as they were formed, or merely mechanically, as appeared to be the case with the amylases.

6. **General comparison of the results of the artificial digestion experiments.** — The action of ptyalin, diastase, and amylopsin in dilute solutions was seriously impaired even by small quantities of bone

ash. In comparatively large proportions bone ash retarded the action of each of these enzymes for many hours or inhibited it in each case altogether. Peptic digestion was retarded somewhat by large amounts of bone ash, apparently because of consequent decrease in the amount of acid. Small quantities of bone ash had very little influence on the peptic process. Tryptic digestion was not disturbed, but, on the other hand, seemed to be favored somewhat by the presence of bone ash, probably as a result of the increased alkalinity imparted by the bone ash. Lipase hydrated milk fats readily in the presence of bone ash. Even relatively large amounts of bone ash did not appreciably retard the lipolysis.

IV. SUMMARY OF GENERAL CONCLUSIONS.

Moderate quantities of bone ash mixed with the food of dogs failed to affect perceptibly the reaction or the specific gravity of the urine, but increased the bulk, and frequency of elimination, of the feces. Such doses of bone ash appeared to reduce the volume of the urine by about the volume of the extra amount of water that was eliminated at the same time in the correspondingly greater bulk of feces. Diarrhea was immediately greatly reduced or stopped entirely by dosage with bone ash.

Moderate doses of bone ash failed to affect appreciably the elimination of total urinary inorganic matter, but the ash from the feces was correspondingly increased in amount. Calcium elimination by the kidneys was unaffected by the bone ash treatment, but the excretion of phosphate in the urine was slightly diminished thereby.

General protein metabolism, as registered by the nitrogen balances for successive periods, was not materially affected by the amounts of bone ash administered in these experiments. The data for urinary sulphur and phosphorus were practically in accord with this conclusion. Urinary nitrogen and phosphorus were decreased somewhat during the dosage periods, whereas the amounts of these elements in the feces were increased in equivalent absolute amounts during the same periods.

Although protein assimilation was perhaps slightly diminished, the reduction, on the most radical assumption the data would permit, must have been trivial. Absorption of both fat and protein seemed to be unaffected and the excretion of bile unmodified. The elimination of mucus appeared to be increased slightly by mechanical influences. Intestinal putrefaction was perceptibly diminished by the bone ash dosage, doubtless as a result of the more frequent evacuations of the bowels that were induced.

Although the action of various amylases was seriously affected by bone ash in the artificial digestion experiments, the two proteases that were studied *in vitro* were not unfavorably influenced when contained in solutions of normal reaction. Tryptic action seemed to be quickened by the bone ash. Lipase likewise was rendered more active *in vitro,* if it was at all affected by the ash.

The results of the artificial digestion experiments warrant the hypothesis that large quantities of bone ash do not unfavorably influence the intestinal enzymotic processes, and that such doses of bone ash have little or no effect on gastric digestion. Such a hypothesis regarding the normal gastro-intestinal digestive processes is further emphasized by the observation that none of the many dogs subjected to bone ash dosage in this laboratory ever indicated any symptoms of indigestion.

The observations recorded by Steel and Gies [19] in some of these connections have been confirmed in every instance.

The use of bone ash in the diet of dogs seems to offer no metabolic disadvantages whatever. The advantages in its use have been referred to in the introduction to this paper and were mentioned in some detail by Steel and Gies.

In conclusion I desire to acknowledge my gratitude to Dr. William J. Gies for his counsel and guidance throughout this work, and to whose interest and advice I owe its completion.

[19] STEEL and GIES: *Loc. cit.*